Title: Tensiomyographic assessment of muscle contractile properties in 9- to 14-year old children

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ABSTRACT

While there are numerous data on the skeletal muscle fibre type composition in adults, little is known about the changes in fibre type composition and contractile properties during maturational growth in children. Using noninvasive tensiomyography we measured contraction time, an indirect estimate of the myosin heavy chain I (MHC-I) proportion, to assess the longitudinal changes of the biceps brachii (BB), biceps femoris (BF), vastus lateralis (VL), and erector spinae (ES) muscles in 53 boys and 54 girls. The children were 9 years at the start of the study and returned for 5 follow-up measurements till the age of 14 years. The ES has the shortest and the BF has the longest Tc. The VL and ES of boys have shorter Tc than those from girls. When applying the relationship between proportion of MHC-I and Tc established in adults to the children TMG data, we found a slow-to-fast transition in the VL between at least the age of 6 and 10 years, when it had stabilized to adult proportions. Regular participation in sport was associated with a faster BF, but not in VL. Our data represents a first non-invasive indication of the developmental changes in muscle fiber type composition in children.

Key words: Contraction time; Muscle Composition; Sport; TMG; Biopsy

What are the new findings:

- The contraction time of skeletal muscles depends on age, sex and it is muscle specific;
- There is a slow-to-fast transition in the vastus lateralis MHC-I between at least the age of 6 and 10 years;
- Regular participation in sport was associated with a shorter contraction time in biceps femoris, but not in vastus lateralis.

INTRODUCTION

Skeletal muscle is indispensable for locomotion, maintenance of body posture and health. To realize locomotion the muscles have to produce power, by generating force and shortening at the same time. Fast fibres can generate about three times as much power as slow fibers of a given size, and this is largely attributable to their 3-times higher maximal shortening velocity, while the force per cross-sectional area does not differ much, if at all, between fibre types[14]. The speed of muscle contraction is largely determined by fibre type composition. The performance of sprinters is therefore helped by a large proportion of fast type II fibres and that of marathon runners by a large proportion of type I fibres. In children, knowing the fibre type composition may be used to help in formulating an informed advise of taking up a sport in which they most likely will excel.

While there are numerous data on the fibre type composition of skeletal muscle in adults and adolescents, we are aware of only seven cross-sectional studies on the fibre type composition of muscle from children between the age of 2 months to 11 years [1,15,20-22,26,39]. Only three of the studies obtained samples from the same skeletal muscle (vastus lateralis-VL) [1,15,21] in the population aged from 6 to 27 years, where [1] reported for 6-year olds that the percentage of type I fibers was similar to that in the VL from adults involved in endurance training. While the VL is the most commonly studied muscle, other muscles are as important in childhood for coherent posture, coordinated motor development, sport performance and health. The other few studies [20-22,26,39] investigated different muscles, making a systematic evaluation of changes in the fibre type composition of a given muscle during early maturation almost impossible. We know of only one longitudinal study that examined the changes of VL composition from childhood to adulthood [15] and hence detailed information of longitudinal changes in fibre type composition of VL and in other muscles during maturational growth are not available.

One of the main causes of the lack of information on changes in fibre type composition during maturational growth is related to the fact that this information is usually obtained from invasively obtained skeletal muscle biopsy samples. Such samples are difficult to obtain from healthy children due to ethical issues, let alone taking repeated samples during maturation from a range of muscles in the same child. These considerations were among the main reasons to develop tensiomyography (TMG), a non-invasive approach to measure skeletal muscle contractile parameters [37,38], that indeed appeared to predict 87% of the variance of the proportion of type I myosin heavy chain in the VL of adults, where the contraction time (Tc) alone accounted for 77% of the variance [33].

Therefore, the objectives of our study were to compare (i) the Tc between VL, long head of biceps femoris (BF), erector spinae (ES), and biceps brachii (BB) muscles; ii) sex-related differences in Tc in abovementioned muscles; (iii) to assess longitudinal age-related changes in the myosin heavy chain 1 (MHC-1) proportion of the VL using our previously developed model; and (iv) to assess in a subgroup of children the effect of regular exercise on Tc in VL and BF muscles, using TMG. We hypothesized that longitudinal age trend of VL MHC-1 is in accordance with previous findings obtained by invasive procedures for both sexes and that regular sport exercise decreases Tc.

METHODS

Participants

At the start of the study the children (*n*=257) were 9.0±.5 years old, being selected from the same class and not exactly the same age. Only Caucasian children were included without any history of neuromuscular disorders. The children were recruited from four randomly selected primary schools in three of the most populated regions of Slovenia. All third-grade children were invited to participate in the study. After a presentation, about 60% parents of the invited children agreed to participate in the study. Informed consent was obtained from the parents of all individual participants included in the study. All procedures meet the ethical standards of this journal [17] and were performed in accordance with the ethical standards of the National Medical Ethics Committee of the Republic of Slovenia and with the 1964 Helsinki declaration and its later amendments.

Initial and five follow-up measurements took place in approximately yearly intervals, being in the range of 242 to 304 days, only between 3rd and 4th measurement was 511 days. The data presented here included only those children who completed all six measurements; this was the case in 107 children (53 boys; 54 girls), average age 9.1±.5 years (Table 1).

Table 1:

Study design

In the longitudinal research design we repeated the measurements on the children during their progression from the third to the eighth primary school grade, six times. We followed the same procedure during each session. One week before each measurement, we notified each school and asked them to follow a specific protocol; no major physical or sport activity should be performed 2 days before the measurement and all children had to be available for the measurements. In each child, we first measured body height and mass, followed by TMG measurements and a short questionnaire.

Tensiomyography

TMG is a non-invasive, selective, and easy to administer tool, which measures muscle belly enlargement in transversal plane during an isometric twitch contraction with a means of digital high-precision displacement sensor to assess the mechanical response of superficial muscles [37]. The main advantage of TMG is selective assessment of skeletal muscle mechanical response to electrical stimuli that encompasses intrinsic properties of muscle belly contraction [31]. TMG is a mechanomyographic method with main distinction – TMG sensor applies 0.2N/cm2 pre-tension on the muscle belly before the measurement is performed [38] to assure high signal-to-noise ratio [31], high reliability [32] and validity to assess MHC-I proportion [33].

TMG was done on the muscles of the dominant leg or arm: BB, ES, VL, and BF in isometric conditions and took approximately 20 minutes. The measurements on the VL were performed supine at 30° knee flexion, while on the BF was performed prone at 5° knee flexion. The measurements on the BB were performed sitting at 90° elbow flexion, arm pronation, and at 0° shoulder flexion, rotation, and adduction. Measurements on the ES were performed prone at 0° hip flexion, arms resting in parallel with the body and head facing downwards.

All muscles were relaxed before and after the measurement (twitch contraction). The oscillations of the muscle belly in response to an electrically-induced twitch were recorded on the skin surface using a sensitive displacement sensor (Linear digital comparator, TMG–BMC, Slovenia), at a 1-kHz sampling frequency. To assure high between day reproducibility of the TMG measurements we followed strict protocol of sensor and electrode placement as well as joint angles standardization (using standard support pads), limb fixation techniques, assuring resting muscle tone by visual inspection and palpation, and maximal twitch stimulation amplitude. The sensor was perpendicular to the skin overlying the muscle belly: in the VL at 30% of femur length above the patella on the lateral side; in the long head of BF at the midpoint of the line between the fibula head and the ischial tuberosity; in BB at 40% of the humerus length above the radius head on the lateral side; and in ES (longissimus part) at the height of the iliac crest.

To elicit a twitch contraction we applied a single 1 ms pulse through the self-adhesive cathode and anode that were placed 5 cm distally and 5 cm proximally to the measuring point, respectively. The stimulation current amplitude at the start was just above the contraction threshold and was then gradually increased until the amplitude of the TMG response did not increase further. Two maximal twitch responses were recorded and saved.

From every twitch response the maximal displacement amplitude (Dm), delay time (Td), contraction time (Tc), and relaxation time (Tr) were calculated as proposed by Valencic [37,38]. The Dm (in mm) was defined as the peak amplitude in the displacement-time curve of the TMG twitch response. Td (in ms) was defined as the time between the electrical stimulus and displacement of the sensor to 10% of Dm; Tc (in ms) was the time between 10% and 90% Dm; and Tr (in ms) was the time from 90% Dm to decline to 50% Dm in the relaxation phase. The average value extracted from two twitch responses was used for further analysis.

Proportion of VL MHC-1 estimation

The proportion of MHC-1 in VL was estimated using the multiple linear regression model [33]. Using three TMG parameters as predictors (Td, Tc, and Tr) we calculated the MHC-1 proportion (Equation 1):

$$\mathrm{MHC{-}1}[\%] = 2.980 \cdot \mathrm{Td} + 2.829 \cdot \mathrm{Tc} + 0.127 \cdot \mathrm{Tr} - 121.023 \qquad [1]$$

Furthermore, the obtained MHC-1 values in the VL of our participants were compared to those reported by others obtained with muscle biopsies [1,15] to extend and compare age- trends.

Sport participation assessment

A short questionnaire was used to obtain information about the out-of-school sport participation of the children. Twenty-nine boys were sporters and 17 non-sporters, while in girls 27 were sporters and 17 non-sporters. Sporters were members of the same sport clubs with at least three hours per week of organized exercise over at least the last 5 years. Children that were not members of sport clubs during a 5-year period were considered non-sporters. A comparison between sporters and non-sporters was performed for VL and BF muscles. Furthermore, we extrapolated our data in both groups with TMG data to adult values and compared those with data seen in the adult sedentary population [32], sprinters [27], dancers [42], volleyball players [29], and football players [28].

Statistical analysis

All data are expressed as means ± standard deviation. For all variables the hypothesis of a normal distribution was tested with visual inspection and the Shapiro-Wilk's test. The effects of age, sex, and muscle on Tc were tested with a three-way General linear model with repeated measures (RM GLM), with age (6 levels) and muscle (4 levels) as within factors and sex (2 levels) as between factors. The effects of age and sex on the estimated MHC-1 proportion in the VL were tested with a two-way RM GLM with age (6 levels) as within and sex (2 levels) as between factor. If age was a significant factor then a Bonferroni corrected post-hoc test was used. If there was a significant age x sex interaction, indicating that age does have different effects in boys and girls, a one-way ANOVA was used to locate those differences. A regular sport effect on the Tc of the VL and BF was evaluated in a subgroup of children using a four-way RM GLM, with age (6 levels) and muscle (2 levels) as within factors and sex (2 levels) and sport (2 levels) as between factors. We excluded 3-way interactions in the analysis. Partial eta-squared (η^2) was used to estimate the effect size after showing significance at at $P < .05$ level. η^2 values were interpreted as low when below .02, medium if between .02 and .13, and large if above .26.

RESULTS

Both boys and girls showed a progressive increase in body height ($P<.001$) and body mass ($P<.001$). The age x sex interactions for body height ($P<.001$) and body mass ($P=.028$) are reflected by a larger increase in body height and body mass in boys than girls (Table 1).

A three-way RM GLM revealed main effects of age ($P<.001$; $\eta^2=.182$), muscle ($P<.001$; $\eta^2=.842$), and muscle x sex ($P<.001$; $\eta^2=.093$) and muscle x age ($P<.001$; $\eta^2=.109$) interactions on Tc. There was no age x sex interaction and therefore for further analysis each of the muscles was analysed separately, excluding sex x age interactions. A sex effect was found in BB ($P=.003$; $\eta^2=.082$), VL ($P=.027$; $\eta^2=.046$), and BF ($P=.004$; $\eta^2=.077$), but not in ES. This was reflected by a longer Tc in the BB and VL in boys than girls and a shorter Tc in the BF of boys than girls (Fig 1). There were also significant age-effects for the BB, VL, BF and ES (all $P<.001$). Post-hoc analysis revealed that the Tc was higher after the age of 11 years in the BB, was decreased between 9.1 and 9.9 years of age in the VL, was increased in the BF after the age of 9.9 years and was transiently reduced in the ES at the age of 10.6 years (Fig 1).

Figure 1:

We found an effect of age ($P<.001$; $\eta^2=.126$) and an age x sex interaction ($P=.043$; $\eta^2=.021$) on the MHC-1 proportion in VL as calculated with equation 1 (Fig 2). Post-hoc analyses revealed a decrease in the proportion of MHC-1 between the age of 9.1 and 9.9 years ($P<.001$) and a higher proportion of MHC-I in boys than girls after the age of 12 years ($P<.05$; Fig 2).

Figure 2

After dividing children into a sport and non-sport group we found an effect of age ($P<.001$; $\eta^2=.110$), muscle ($P<.001$; $\eta^2=.902$), age x sport ($P=.024$; $\eta^2=.030$), age x sex ($P=.021$; $\eta^2=.030$) and muscle x sport interactions ($P<.001$; $\eta^2=.168$) on Tc of the BF and VL. Post hoc analysis revealed no sport effect on Tc in the VL, while in the BF Tc was longer in non-sporters than sporters after the age of 12 years in boys and girls (Fig 3).

Figure 3

DISCUSSION

The main findings of our study are that: (i) the BB and VL muscles of boys have higher Tc than that of girls, while the opposite applies to the BF; (ii) in both boys and girls the VL and ES muscles develops with shorter Tc before the onset of puberty to then stabilize at levels similar to that observed in adult muscles; (iii) regular participation in sports was associated with shorter BF Tc in both boys and girls, while there was no effect of sport participation on the VL Tc. This study thus suggests that during normal prepubertal maturational growth, that was the case also in our study [30], skeletal muscles in healthy children become faster. Especially, where it is very difficult, for ethical reasons, to measure directly skeletal muscle MHC or fibre type composition, TMG provides non-invasive information on changes in functional properties in skeletal muscle during maturational growth.

TMG is a mechanomyographic method that uses displacement sensor to detect the bulging of the muscle belly during muscle contractions [38]. Using a mathematical-mechanical model for viscoelastic properties of muscle concluded that damping of the signal is on average for 4.6±3.2 times higher through the longitudinal pathway (torque) than the transversal pathway (TMG), causing a 42.7% delay in the peak of the twitch response during torque measurements compared to that detected with TMG [31]. It was therefore suggested that TMG is more suitable to estimate the intrinsic Tc of the muscle. In line with this, previous studies reported a positive correlation between Tc, measured with TMG, and the proportion of type I muscle fibres [5,7] and MHC-I in VL [33] and suggests that TMG can be used to give an estimate of muscle fiber type or MHC-I proportions, at least in the VL muscle.

Differences between muscles

Using TMG, we found that the ES had the shortest Tc, and hence was the fastest muscle, followed by VL, BF, and BB. A shorter Tc in VL than in BF has also been found in adults [5,7] and corresponds with the lower proportion of type I fibres in the VL (surface 37.8%; depth 46.9%) than in the BF (66.9%) [18].

While the differences in Tc between the VL and BF appear to correspond with the difference in fiber type composition between those muscles, this does not apply to the difference in Tc between the VL and ES. While the ES had the shortest Tc, it has a higher proportion of type I fibers (58.4% and 54.9% at surface and depth) than the VL

[18]. Part of the discrepancy may be attributable to the relatively small pennation angle in ES (less than 1.6 degrees) [4,9] in comparison to VL, BF, and BB, where smaller pennation angles result in higher shortening velocities of the muscle [8]. So far, there have been no studies to assess the effects of muscle architecture on TMG-measured Tc.

Sex-related differences

We found no difference in the Tc of the ES between boys and girls. Boys, however, had a shorter BF Tc than girls. This would augment the better anaerobic performance in actions where BF is predominantly utilized – e.g. sprints and jumps [23,36] in boys than girls, especially after the age of 12 years.

The BB and VL have, however, higher Tc in boys than girls, which corresponds with the higher proportion of type I fibers in the VL reported in 16-year-old boys than girls [15]. This may shift during puberty and adolescence; however, as in follow up measurements when the participants were 27 years old the MHC-1 proportion had become less than in the girls [12]. In this context, it is interesting to note that performance in sprints or jumps [23,36] and in peak anaerobic cycling [12] of boys starts to exceed that of girls after the age of 12 and 14, respectively. While this is undoubtedly largely attributable to their larger muscle mass, first evident at the age of 12 years [41], the increased contractile speed beyond that age will further enhance performance. Only two studies reported comparison between TMG parameters of both sexes, where female adult kayakers have similar Tc as male kayakers [13], while female adult dancers have shorter Tc in latissimus dorsi and quadriceps muscles and longer Tc in triceps brachii and gastrocnemius muscles [42]. These sex-related differences are expected due to sport specifics, confirming sport participation as an important factor of skeletal muscle contractile parameters.

Age-related differences

The most intriguing age-related observation is the decrease in Tc in the VL and ES between the age of 9.1 and 9.9 years in both boys and girls, indicating that the muscles became faster in this period. This decrease could be related to a spinal growth spurt that occurs around that age [10,11], that is associated with loss of muscle flexibility [24], but it remains to be seen how this could translate into a shorter Tc.

In the ES, a subsequent gradual slowing until the age of 13.6 years followed this. Combining the observations in the VL with a study on the fiber type composition of the VL in 6-year-old children [1] indicates that this slow-to-fast transition in the VL was even more pronounced between the age of 6 and 9.1 years. We did not see such a slow-to-fast transition in the other muscles, and also in the triceps surae no evidence for such a fiber type transition was found, as reflected by the similar twitch contraction time in 7 to 11-year-old children [16].

The Tc in BB increased between 10.6 and the age of 12 years. In comparison to data in adults [40], the BB of children has a longer Tc, reflecting slower muscles, than that in adults. This is confirmed also with biopsy data where children (3-7 years) have higher proportion of type I fibers in the BB than adults (54% *vs.* 42%, respectively) [18,19,26].

Our data indicates shorter Tc of VL, while longer Tc in BB of children in comparison to adults. A possible mechanism could be in lower motor-unit activation level of predominantly type 2 fibres in boys (78%) than in men (95%) in the knee extensors, but not in the elbow flexors [2]. Speculating, lower activation level confirms higher proportion of inactive type 2 fibres and inactivity leads to higher velocity of contraction of single fibres [3].

As there are no data on fiber type composition of the ES and BF in children and only limited data for VL and BB muscles, our data represents a first non-invasive indication, although only indirectly, of the developmental trends in changes in muscle fiber type composition in children.

Sport-related differences

We found that sport participation was associated with a lower Tc in the BF muscle (reflective of a reduction in the proportion of MHC-I) in both boys and girls after the age of 12 years, while no such effect was seen in the VL. A similar situation was seen in adult track and field sprinters where sport participation resulted in a higher proportion of type IIc fibres in the BF, which was also associated with a lower Tc (19.5 vs. 30.2ms, in sprinters vs. sedentary) [6]. It could be that the load on weight bearing muscles from normal daily physical activities in children is already relatively high in non-sporters and that the non-weight bearing muscles get challenged more during sport participation. If so, this may explain the larger adaptation to regular exercise in the BF than the VL. When we compare this with specific adult populations (Fig 3) we see that the response may be sport activity specific as the Tc of male sprinters is 19 ms [27], 25ms in volleyball players [29], 27ms in gymnasts [35] and football players [28], 32 ms in non-athletes [32] and that of dancers 34 ms [42]. It thus appears that participating in sport as a child may result in a faster profile of the BF, an important muscle for fast explosive sports e.g. football, volleyball, sprint, and gymnastics. To support this, we previously reported that children that were involved in regular sport exercise have also higher sprinting velocity [40] and that was negatively correlated to BF Tc [26,43]; however, only in boys after the age of 13 years.

Conclusion

In conclusion, we found with TMG that pre-pubertal to early-pubertal boys had in general slower muscles than girls. During early maturation in the VL there is a slow-to-fast transition between at least the age of 6 and 10 years, that then appears to stabilize to adult proportions. Regular participation in sport was associated with a faster BF but not VL. Our data thus represent a first non-invasive, although indirect, indication of the developmental trends in changes in muscle fiber type composition in children.

Study limitations

The participants were initially recruited from the same class and were not exactly the same chronological age. The multiple linear regression model for estimating the VL MHC-1 (Equation 1; [33]) was developed on 27 participants aged between 20 and 83 years. Here we used it to examine our sample of children from 9.1 to 13.6 years of age. Although this approach has not been validated in children, there are no obvious reasons to believe that it would not equally well apply to muscles of children. In fact, comparison with the literature reveals close similarities with reported fibre type compositions derived from biopsies and in most cases known differences in fibre type composition between muscles were reflected by qualitatively similar differences in Tc. We controlled for maximal stimulation amplitude by assuring resting muscle tone; however, we did not applied supramaximal stimulation amplitudes due to ethical reasons.

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